energy and 72.72 kJ/M energy was used in case of osteoarthritic samples. Loss of water content in all three groups are presented with a sharp step on the TG curve, starting on average temperature of 37 °C and ending at 116 °C. Linear part of the TG curve begun at around 62 °C and ended at around 112 °C. loss. In case of the normal hyaline cartilage 1.266%/1°C fluid loss was detected. In necrotic samples 1.689%/1 °C decrease in mass was observed. In the osteoarthritic 1.422%/1 °C mass reduction was measured

Conclusions: Increase in the cartilage matrix water content in all cases of degenerative articular cartilage was observed. Based on the results it can be stated that water content is higher in impaired samples, meanwhile water interstitial bonding was stronger in these cases. Activation energy correlated considerably with water content in the samples. The newly established thermogravimetric protocol was sufficient for compositional thermoanalytical study of normal and degenerative human hyaline cartilage. Previously, this method has not been used for this purpose. Characterization of the altered metabolism in cartilage that promote disease progression should lead to future treatment options that can prevent structural damage. Therapeutic steps can be adequately tested and monitored with thermogravimetric measurements.

P191

EVIDENCE FOR CLEAVAGE OF TYPE II COLLAGEN BY CATHEPSIN K IN HUMAN OSTEOARTHRITIC CARTILAGE

V.M. Dejica, J.S. Mort, A.R. Poole
Shriners Hospital, Montreal, PQ, Canada

Purpose: Cathepsin K is expressed in normal and osteoarthritic (OA) hyaline cartilage and is capable of cleaving type II collagen as well as other matrix molecules. The aim of this study was to determine whether there is evidence for cathepsin K- mediated cleavage of type II collagen in human OA cartilage.

Methods: Femoral condylar cartilages removed at arthroplasty for knee OA were cultured in serum-free medium in the presence and absence of a synthetic cathepsin K inhibitor (supplied by Merck Frosst, Montreal, Quebec, Canada). The content of a new type II collagen cleavage neoepitope that can be generated by cathepsin K was measured by ELISA assay. Aggrecan degradation was measured by the release of glycosaminoglycan using a colorimetric assay. Inhibitor toxicity was assessed by measuring the incorporation of [^{3}H] proline in cartilage cultured with and without the inhibitor. Type II collagen cleavage was also detected by ELISA and immunohistochemically in uncultured cartilages from both normal and OA knee joints.

Results: Cleavage of type II collagen was significantly enhanced in OA cartilage compared with healthy cartilage, as demonstrated by ELISA and immunolocalization. The inhibitor reduced collagen cleavage in cultures of 4 out of 8 patients, this being significant in 3 cases. There was no effect on proteoglycan release and the incorporation of tritiated proline was unaffected by the inhibitor.

Conclusions: These results show that cleavage of type II collagen at a site cleaved by cathepsin K is increased in OA articular cartilages. Based on the specificity and lack of detectable toxicity of the inhibitor, this cleavage is due in part to cathepsin K in almost half of the patients. Cathepsin K should therefore be considered as a potential therapeutic target in the control of cartilage degeneration in OA.

P192

BOVINE, PORCINE AND ICHTHYIC CHONDROITIN SULFATE DECREASE IL-1beta EFFECTS ON NO PRODUCTION AND APOPTOSIS: CORRELATION WITH MOLECULAR MODELING DATA

F. Heraud[1], F. Burger[1], E. Rebuffet[2], S. Amigues[1], C. Soler[1], A. Imberty[2]
[1]Laboratoires Genevrier, Sophia Antipolis, France, [2]CERMAV-CNRS, Grenoble, France

Purpose: The current study examines whether porcine, bovine and ichthyic chondroitin sulfate (CS) would influence the production of nitric oxide (NO) and apoptosis in human osteoarthritic (OA) chondrocytes. Then, we confirm these results and explain them by a proposed novel activity concept of CS evaluated by molecular modeling.

Methods: Samples of human OA articular cartilage were obtained from patients undergoing knee arthroscopy. Firstly, OA human chondrocytes were incubated with porcine, bovine and ichthyic CS (100μg/mL) and stimulated with human recombinant Interleukin-1 beta (hrIL-1 beta) (10ng/mL), in the same time, to induce NO synthesis. NO release was measured as nitrite concentration in 24 and 48 hours culture supernatants by using the Griess reaction. Secondly, OA human chondrocytes were incubated with porcine, bovine and ichthyic CS during 72 hours and stimulated by various concentrations SNP (Sodium Nitroprusside) during 18 hours. SNP was used as a NO compound donor. To access the degree of apoptosis, APOPercentage Apoptosis Assay was used. This finding was further quantitatively confirmed by fluorescent microscopy using two apoptosis markers: TUNEL assay and Annexin-V fluos. In the third time, we modelized several CS oligosaccharides and we have tested their possibilities of interaction with IL-1 beta and its receptor.

Results: Bovine, porcine and ichthyic CS tested decreased significantly NO synthesis at 48 hours when human OA chondrocytes were cotreated with CS and hrIL-1beta. However, a preventive treatment with bovine, porcine and ichthyic CS during 72 hours and a stimulation with hrIL-1beta did not reduce significantly NO synthesis.

In OA chondrocytes treated with bovine, porcine and ichthyic CS, on average 18% of chondrocytes showed apoptotic features compared with 31% in chondrocytes treated with SNP. These data suggest that CS or CS oligosaccharides could interact with IL-1beta. The modeling study proposed two sites of interactions between IL-1 beta and some oligosaccharides. We have probed the specificity of this protein for distinct sulphatation sequences.

Conclusions: These results suggest that bovine, porcine and ichthyic CS prevent IL-1beta induced increase in NO production. This preliminary study suggests that bovine, porcine and ichthyic CS could downregulate apoptosis in the OA chondrocytes. A decrease of IL-1 beta effects could be the consequence of specific binding to oligosaccharides.

This study provides a plausible mechanism for the chondroprotective properties of bovine, porcine and ichthyic CS.

P193

CHONDROCYTE HYPERTROPHY - A NOVEL EX VIVO MODEL FOR EARLY CHANGES IN CHONDROCYTES IN OA

B.-C. Sondergaard[1], S.H. Madsen[1], P. Qvist[1], C. Christiansen[2], M.A. Karsdal[1]
[1]Nordic Bioscience Diagnostics, Herlev, Denmark, [2]CCBR, Ballerup, Denmark

Purpose: In early osteoarthritis (OA), hypertrophic chondrocytes are part of the pathology and are distributed throughout the